Phenolic compounds profile of water and ethanol extracts of *Euphorbia hirta* L. leaves showing antioxidant and antifungal properties

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Abstract

The bioactive chemical constituents of water and ethanol extracts of *Euphorbia hirta* L. leaves have been identified and quantified using an un-targeted mass spectrometric approach. The study allowed the tentative identification of 123 individual phenolic compounds and 18 non-phenolic phytochemicals, most of them described in *Euphorbia hirta* L. leaves for the first time. Gallotannins, hydroxybenzoic and hydroxycinnamic acids were the most abundant phenolic classes in *Euphorbia hirta* L. leaves, representing together the 71.5% (26.3%, 25.2% and 20%, respectively) of the total amount of identified phenolics. The main phenolic compounds detected were tri-*O*-galloyl-glucose isomers, feruloyl-coniferin, tetra-*O*-galloyl-glucose isomers, di-*O*-galloyl-glucose isomers, ethyl-gallic acid, protocatechuic acid-*O*-pentoside-*O*-hexoside, 5-*O*-caffeoyl-quinic acid *trans* isomer and digalloyl-quinic acid. Feruloyl-coniferin was found to be approximately six times more concentrated in the ethanol extract with respect to the water extract. The ethanol extract exhibited higher ABTS (1338.3 ± 85.3 and 802.3 ± 91.0 μmol ascorbic acid equivalent/gram of dry extract, respectively) and superoxide anion (2014.6 ± 78.6 and 1528.0 ± 111.7 μmol ascorbic acid equivalent/gram of dry extract, respectively) scavenging abilities than the water extract. Additionally, the ethanol extract also showed a remarkable anti-fungal effect against *Fusarium oxysporum* f. sp. *vasinfectum*, *Alternaria solani* and *Rhizoctonia solani.* This study provides new information about *Euphorbia hirta* L., offering reasons to promote this plant species as rich source of phenolics and an excellent source of antifungal molecules that might have a prospective use in controlling fungal diseases of vegetable crops.

Keywords: Bio-fungicides; phytochemicals; mass spectrometry; phytopathogenic mycetes; tomato.

1. Introduction

Euphorbia hirta L. (*E. hirta*) is a plant species commonly found in all tropical countries worldwide, including Cameroon. *E. hirta* belongs to the spurge family of *Euphorbiaceae*. Although it can be seen lying down sometimes, it is usually upright, slender-stemmed, spreading up to 80 cm tall **(**Abu et al., 2011).

E. hirta is a very popular medicinal herb and has been used since ancient times as decoction or infusion in traditional remedies to treat gastro-intestinal diseases and disorders (*e.g.* intestinal parasites, diarrhoea and peptic ulcer), skin problems and asthma (Huang et al., 2012). More recently, extracts from *E. hirta* have shown a broad range of biological properties, including antimicrobial, antifungal, anti-inflammatory, antioxidant, anticancer and antidiabetic activities (Almosnid et al., 2018; Kumar et al., 2010; Li et al., 2015). Several phytochemicals have been already extracted and identified from *E. hirta* leaves, such as terpenoids, coumarins, lignans and phenolic compounds (Kumar et al., 2010; Li et al., 2015; Yi et al., 2012). The latter components, widely known for their antioxidant and biological activities, have been rarely investigated. In this context, previous phytochemical studies showed that the leaves from *E. hirta* were characterized by the presence of flavonols (quercetin and myricetin derivatives, and kaempferol), hydroxybenzoic acids (gallic and protocatechuic acids), tannins (gallotannins and euphorbins), flavones (luteolin) and lignans (pinocembrin, pinoresinol derivatives and syringaresinol derivatives) (Kumar et al., 2010; Li et al., 2015; Yi et al., 2012). However, a comprehensive identification and quantification of the phenolic profile of *E. hirta* leaves is still lacking.

Phytopathogenic fungi are the causative agents of several important diseases of cultivated plants, responsible of enormous crop losses in agriculture (Eloff et al., 2017). In this context, the application of chemical fungicides is the most widespread pest management strategy to prevent yield and quality losses. Quite frequently, the development of resistance traits among the pathogens is the result of massive and improper use of these chemicals (Lucas et al., 2015). Moreover, some of

these fungicides may seriously affect human health due to the environmental pollution and the presence of residues frequently detected in fruits and vegetables.

Currently, the research on alternative natural products with potential use in pest management strategies is very active (Bocquet et al., 2018; Eloff et al., 2017; Wu et al., 2018). To this purpose, phenolic-rich plant extracts were shown to display antifungal activity against different pathogenic fungi, including *Fusarium oxysporum*, *Rhizoctonia solani*, *Phytophthora nicotianae*, *Alternaria alternata* and *Aspergillus* species (Eloff et al., 2017; de Rodríguez et al., 2015; Wu et al., 2018). In addition, purified phenolics demonstrated a direct antifungal action as well, such as ferulic acid against *Botrytis cinerea* or alkylresorcinols against different *Fusarium* spp. (Patzke et al., 2017; Patzke and Schieber, 2018).

The aim of the present study was to identify and quantify the phenolic profile of two different extracts (water and ethanol extracts) of *E. hirta* leaves by using an un-targeted mass spectrometry approach. The two different extracts were also characterized for their antioxidant properties and their ability to inhibit the growth of some plant pathogenic fungi affecting tomato.

2. Materials and methods

2.1. Chemicals and reagents

Phenolic standards (quercetin, quercetin-3-*O*-rutinoside, quercetin-3-*O*-glucoside, kaempferol, epicatechin, ellagic acid, gallic acid, protocatechuic acid, caffeic acid, *p*-coumaric acid, and ferulic acid) and reagents for analytical determination were purchased from Sigma-Aldrich (Milan, Italy). Deionized water was obtained from a Milli-Q System (Bedford, MA, USA). The mass spectrometry reagents and solvents for phenolic compounds extraction were obtained from BioRad (Hercules, CA, USA).

2.2 Fungal strains

Three pathogenic fungi affecting tomato were used: *Fusarium oxysporum* f. sp. *vasinfectum* Snyder et Hansen, strain FUSITS04 (from Cameroon), *Alternaria solani* Sorauer, strain ASU4 (from Cameroon) and *Rhizoctonia solani* Kuhn, strain RsG1 (isolated in Italy and kindly provided by Paola Nipoti, University of Bologna). The fungi were maintained on 3.9% (m/v) potato dextrose agar (PDA) medium at 27°C until their use.

2.3. Plant material

A tropical plant species, *Euphorbia hirta* L. (family: *Euphorbiaceae*), native and widely spread in Cameroon was selected for the production of the extracts. Plants were collected from a local area (Central Region, Yaoundé-Mbankomo, Cameroon) and identified by Tadjouteu Fulbert by comparison with the botanical collection of A. J. M. Leeuwenberg, number 10480 and registered at the Cameroon National Herbarium in Yaoundé under the number 48982/HNC. Whole plants, including roots, were harvested just before the flowering stage (**Figure S1**).

2.4. Preparation of crude extracts

Water and ethanol plant extracts were obtained as described in Nguefack et al. (2013). After manual harvesting, the whole plants were shade-dried at a temperature of 35°C for 15 days. Dry leaves were detached from plants and then milled into powder using a GRAIN MILL MAGNUM 4V (motor power: 1 HP 750 Watt, 13,000 to 15,000 rpm). Aliquots of 100 g of plant powder were first defatted by mixing with 600 mL of hexane on a rotary shaker at 120 rpm for 24 h at room temperature. After filtration with a fine cloth, the plant residue was spread on an aluminium foil under a sterile hood, allowing the complete hexane evaporation. Lipid-free dry powder was then used for the extraction. Two solvents were simultaneously used in two different extraction procedures: distilled water and 70% ethanol/water solution. For both extractions, the defatted plant material was soaked and stirred in 600 mL of distilled water or, alternatively, 70% ethanol for 24 h at room temperature, followed by filtration through Whatman N°1 filter paper. The resulting filtrates were then centrifuged at 5,200×*g* for 10 min and the supernatants were evaporated into a ventilate oven at 50°C overnight to obtain dried pellets. Dried pellets were named water extract and ethanol extract. The average yields were 8.20% for water extract and 5.60% for ethanol extract.

2.5. LC-ESI-IT-MS/MS analysis of phenolic compounds

For LC-MS/MS analysis, 20 mg of powders from water and ethanol extracts of *E. hirta* leaves were re-suspended in 1 mL of the respective solvents (water and 70% ethanol, respectively). The extracts were then analysed on a HPLC Agilent 1200 Series system equipped with a C18 column (HxSil C18 Reversed phase, 250×4.6 mm, 5 µm particle size, Hamilton Company, Reno, Nevada, USA) as reported in Mena et al. (2016). The mobile phase consisted of (A) H_2O/formic acid (99:1, *v*/*v*) and (B) acetonitrile/formic acid (99:1, *v*/*v*). The gradient started at 1% B for 1 min then linearly ramped up to 40% B in 13 min. The mobile phase composition was raised up to 99% B in 13 min and maintained for 2 min in order to wash the column before returning to the initial condition. The flow rate was set at 1 mL/min. The samples were injected in the amount of 40 µL. After passing through

111 the column, the eluate was split and 0.4 mL/min were directed to an Agilent 6300 ion trap mass
112 spectrometer. Two MS experiments were performed, one in ESI negative ion mode and one using
113 positive ESI ionization, under the same chromatographic conditions. Identification of phenolic
114 compounds in all samples was carried out using full scan, data-dependent MS^2 scanning from *m/z*
115 100 to 800.
116 Ellagitannins were quantified as ellagic acid equivalents whereas gallotannins as gallic acid
117 equivalents. Flavonols were quantified as quercetin-3-glucoside or kaempferol equivalents. Flavan-
118 3-ols were quantified as catechin equivalent. Hydroxybenzoic acids were quantified as gallic acid or
119 protocatechuic acid equivalents whereas hydroxycinnamic acids as caffeic acid or coumaric acid or
120 ferulic acid equivalents. Isocoumarins were instead quantified as gallic acid equivalents.
121 ESI-IT-MS/MS parameters, limits of detection (LOD) and limits of quantification (LOQ) for the
122 different standards were the same as reported in Martini et al. (2017).
123 Quantitative results were expressed as mg of compounds per g of dry extract.
124
125 *2.6 Antioxidant activity analysis*
126 The antioxidant properties of water and ethanol fractions obtained from *E. hirta* leaves were
127 analysed by using four different assays. The samples were obtained by dissolving 20 mg of powders
128 from water and ethanol extracts in 1 mL of the respective solvents (water and 70% ethanol,
129 respectively). The radical scavenging ability was tested by using the ABTS assay according to Re et
130 al. (1999). For the determination of the reducing ability, a protocol based on the ferric
131 reducing/antioxidant power (FRAP) assay was used (Benzie and Strain, 1999). The capacity to
132 scavenge superoxide anion radicals was evaluated according to the methods reported by Martini et
133 al. (2017). The results were expressed as μmol of ascorbic acid equivalent per gram of dry extract.
134 The Fe^{2+}-chelation ability was instead evaluated by the ferrozine assay (Karama and Pegg, 2009).

Re-dissolved water and ethanol extracts of *E. hirta* leaves were diluted 20-fold in the respective solvents and tested for the chelating ability. Results were expressed as percentage of bound Fe^{2+}.

2.7 Antifungal activity

The agar plate dilution method, as described by Rios et al. (1988), was performed to investigate the direct antifungal activity of the extracts. Five increasing dilutions of the plant extracts were used to obtain the following final concentrations: 1.25, 2.50, 5, 10, and 20 mg of dry extract/mL. PDA plates without any addition of plant extracts were used as a negative control.

A 5 mm mycelial disk of each phytopathogenic fungus was placed on the centre of an agar plate and then incubated at 26°C. After 7 days, in order to assess the fungal growth, the two perpendicular diameters of the fungal mycelium were measured. Growth inhibition was calculated comparing fungal growth on pre-treated PDA plates with the growth on PDA without any addition of plant extracts. Growth inhibition percentage (% I) was calculated according to the formula developed by Pandey et al., (1982):

$$\% I = [(MGC - MG)/MGC] \times 100$$

where, MGC = mycelium growth diameter in the control PDA plate, *MG* = mycelium growth diameter in the pre-treated PDA plate.

The concentration of plant extracts required to inhibit by 50% the fungal growth (IC_{50}) was determined by plotting the growth inhibition percentage as a function of final plant extract concentration (base-10 logarithm). IC_{50} values were expressed as mg of extract/mL.

2.8. Statistics

Mass spectrometry and antioxidant activity data are displayed as mean ± SD for three replicates for each prepared sample. Antifungal activity data are reported as mean ± SD for five replicates. Univariate analysis of variance (ANOVA) with Tukey's post-hoc test was applied using GraphPad

prism 6.0 (GraphPad Software, San Diego, CA, U.S.A.) when multiple comparisons were performed. The differences were considered significant with $P < 0.05$. IC_{50} for antifungal activity was calculated by using non-linear regression analysis (GraphPad prism 6.0; GraphPad Software, San Diego, CA, U.S.A.).

3. Results and discussion

3.1. Identification of the major phytochemicals in water and ethanol extracts of Euphorbia hirta leaves

In this study, the water and ethanol extracts of *E. hirta* leaves were analysed for their phytochemical profile. The phytochemical composition of the extracts was investigated using an un-targeted method through LC-ESI-IT-MS/MS experiments. Representative base peak chromatograms (BPCs) are shown in **Figure 1**. This approach allowed the tentative identification of 123 individual phenolic compounds, 7 organic acids, 4 terpenes, 3 amino acids, 1 dipeptide, 1 alkaloid, 1 anthraquinone and 1 norisoprenoid.

Ten compounds were identified by comparison with reference standards, while the remaining 131 compounds were tentatively identified based on the interpretation of their fragmentation patterns obtained from mass spectra (MS^2 experiments) and by comparison with the literature. The mass spectrum data along with peak assignments and retention time for the identified phytochemicals are described in **Tables 1** and **2**.

3.1.1. Ellagic acid derivatives and ellagitannins

A total of 21 ellagic acid derivatives and ellagitannins were identified in the *E. hirta* extracts. Compound **41.1** presented a negative charged molecular ion at *m/z* 285 ($[M-H]^{-1}$) and the same fragmentation pattern as ellagic acid with a base peak at *m/z* 257 and secondary peaks at *m/z* 229 and *m/z* 185 (Calani et al., 2013). However, its *m/z* value was 16 Da lower than that of ellagic acid and was tentatively identified as deoxyellagic acid. Compound **31.5** was identified as ellagic acid by comparison of the retention time and mass spectral data with the authentic standard. Compounds **28.1** and **30.4** showed an *m/z* ion at 519 ($[M-H]^{-1}$) and the typical fragmentation pattern of ellagic acid. The loss of 218 Da is typical of a pentoside-malonyl group (loss of −86 Da and −132 Da corresponding to malonyl and pentoside moieties, respectively) and these compounds were,

therefore, identified as isomers of ellagic acid malonyl-pentoside. Compound **32.5** gave a pseudomolecular ion at *m/z* 601 ($[M-H]^{-1}$) and a fragmentation pattern consistent with gallagic acid (Mena et al., 2012). Ellagitannins are characterized by the presence of a hexahydroxydiphenoyl (HHDP) moiety, which results in the typical appearance of fragment ions at *m/z* 301 and *m/z* 275 in the MS^2 spectra. Compounds **2.2**, **3.1** and **5.1** yielded the same pseudomolecular ion at *m/z* 481 and, on the basis of the fragmentation spectra, they were identified as HHDP-hexoside isomers (Calani et al., 2013). Three signals (compounds **6.7**, **11.1** and **20.1**) at *m/z* 633 were observed and identified as galloyl-HHDP-hexoside (corilagin) isomers (Sousa et al., 2016). Additionally, two signals at *m/z* 631 (compounds **17.2** and **18.1**) showed the same fragmentation pattern of galloyl-HHDP-hexoside. These compounds were identified as dehydro-corilagin isomers. Based on previously published fragmentation spectra (Mena et al., 2012; Calani et al., 2013), compounds **17.1** and **22.2** were identified as pedunculagin II and pedunculagin I, respectively, whereas compounds **21.2** and **27.2** were identified as granatin B isomers. Finally, four signals (compounds **6.3**, **7.1**, **9.1** and **31.4**) displayed the typical fragmentation pattern of ellagitannins, but it was not possible to assign them an exact structure.

3.1.2. Gallotannins

In this study, 11 gallotannins were detected in the *E. hirta* leaves extracts. Gallotannins are polyphenolic compounds with a sugar core linked to at least two gallic acid moieties. The MS^2 spectra of gallotannins usually gave typical fragment ions at *m*/*z* 331, 313 and 169, which correspond to the moiety of galloyl-hexoside, galloyl-hexoside $–H_2O$, and gallic acid, respectively (Gu et al., 2013). The typical losses included gallic acid moieties (152 or 170 Da) and sugar units (162 Da) (Hukkanen et al., 2007). According to the proposed fragmentation pathway, compounds **10.3** and **14.5** were identified as di-*O*-galloyl-glucose isomers (Gu et al., 2013). Compounds **16.1**, **19.2**, **23.2** and compounds **23.4** and **27.3** were identified as isomers of tri- and

tetra-*O*-galloyl-glucose, respectively. The product ion at *m/z* 483 corresponding to the deprotonated di-*O*-galloyl-glucose molecule and originating from successive loss of galloyl groups can be observed in the MS^2 spectra of both tri-*O*-galloyl-glucose and tetra-*O*-galloyl-glucose. The fragmentation of tetra-*O*-galloyl-glucose isomers also generated a signal at *m/z* 635 corresponding to the deprotonated tri-*O*-galloyl-glucose molecule. Finally, compound **30.1** was assigned to penta-*O*-galloyl-glucose. Compound **24.3** had a pseudomolecular ion at *m/z* 467 and produced at MS^2 *m/z* 315 and 169 corresponding to the loss of galloyl group (-152 Da) and galloyl group plus deoxyhexose (-152 and -146 Da). Therefore, this compound was tentatively identified as di-*O*-galloyl-rhamnose. Finally, two signals (compounds **6.4** and **33.1**) displayed the typical fragmentation pattern of gallotannins but it was not possible to assign an exact structure to these molecules.

3.1.3. Flavonols

Among the 34 flavonol derivatives (**Table 1**) detected, 16 compounds were identified as quercetin-derivatives and 13 as kaempferol-derivatives. Quercetin-derivatives can be easily identified by the presence of the typical fragment ions in the MS^2 spectra at *m/z* 301, 271, 179 and 151 derived from the fragmentation of the quercetin aglycone (Fabre et al., 2001). Compound **40.1** was identified as quercetin aglycone by comparison with the authentic standard. Compounds **32.3** and **33.3** presented an identical pseudomolecular ion $[M-H]^-$ at *m/z* 433, releasing a fragment ion at *m/z* 301 (loss of a pentose group), which might be coherent with quercetin-*O*-pentoside isomers. The appearance of the signal at *m/z* 271 (Y_0-2H-CO) characteristic of 3-*O*-glycosyl flavonols in the MS^2 spectra of the compound **32.3** pointed as the existence of a 3-*O*-pentoside binding site and the compound was therefore identified as quercetin-3-*O*-pentoside (Ablajan et al., 2006; Martini et al., 2018). Compound **33.3,** instead, showed the presence of a signal at *m/z* 273 (Y_0-CO), which is characteristic of 7-*O*-glycosyl flavonols, and the compound was therefore identified as quercetin-7-

239 *O*-pentoside (Ablajan et al., 2006; Martini et al., 2018). Compound **33.4** showed a pseudomolecular
240 ion at *m/z* 477 and was identified as quercetin-3-*O*-rhamnoside due to the presence of the signals at
241 *m/z* 301 (quercetin aglycone originating from the loss of the rhamnosyl moiety) and 271.
242 Compound **23.3** had the same negative molecular ion (*m/z* 463) as compound **31.1**, which was
243 identified as quercetin-3-*O*-glucoside by comparison with the authentic standard. The analysis of
244 MS^2 spectra revealed the loss of 162 Da (hexose group) to produce an *m/z* 301 (quercetin) daughter
245 ion. Basing on the elution order, this compound was tentatively identified as quercetin-3-*O*-
246 galactoside (Del Rio et al., 2004). Compound **31.6** presented a pseudomolecular ion $[M-H]^-$ at *m/z*
247 477 releasing a fragment ion at *m/z* 301 (loss of a glucuronide group), which might be coherent with
248 quercetin-3-*O*-glucunoride (Dall'Asta et al., 2012). Compounds **32.4** and **35.1** showed the same
249 negative molecular ion (*m/z* 505), which gave product ions in the MS^2 spectra at *m/z* 463 (loss of
250 acetyl group) and 301 (loss of hexose group). The presence of the peak at *m/z* 271 allowed us the
251 identification of the peaks as quercetin-3-*O*-acetyl-hexoside isomers (Ablajan et al., 2006;
252 Cuyckens and Claeys, 2004). Compounds **33.5**, **35.5** and **37.3** exhibited identical negative
253 molecular ion (*m/z* 585) and peaks at *m/z* 433 (loss of a galloyl group) and 301 (loss of a pentoside
254 group) in the MS^2 spectra. The presence of the peak at *m/z* 273 allowed us the identification of the
255 peaks as quercetin-7-*O*-galloyl-pentoside isomers (Ablajan et al., 2006; Cuyckens and Claeys,
256 2004). Quercetin-3-*O*-rutinoside (compound **29.1**; *m/z* 609) was identified by comparison of
257 retention time and fragmentation spectra with the authentic standard. Compounds **31.7** and **37.2**
258 showed the same pseudomolecular ion at *m/z* 615, which gave product ions in the MS^2 spectra at
259 *m/z* 463 and 301, thus indicating a successive loss of a galloyl group (-152 Da) and a hexosyl
260 moiety (-162 Da). Due to the presence of a peak at *m/z* 271, these compounds were tentatively
261 identified as quercetin-3-*O*-galloyl-hexoside isomers (Ablajan et al., 2006; Cuyckens and Claeys,
262 2004). Compound **14.6** (*m/z* 625) presented peaks at *m/z* 463 (loss of a hexose group), 301 (loss of a
263 second hexose group), 300, 273 and 271 in the fragmentation spectra. The presence of the peak at

m/z 463 (Y_1) is indicative that the two hexosyl groups are attached in different position. The observed peaks at m/z 273 and 271 indicated that one sugar is linked to the -OH group in position 3 and the other one to the -OH group in position 7 of the aglycone (Ferreres et al., 2004; Li and Claeys, 1994). This peak was assigned to quercetin-3-*O*-hexoside-7-*O*-hexoside. Compounds **14.2**, **24.4**, **25.3**, **31.2**, **32.2**, **33.2**, **33.6**, **35.3**, **35.4**, **36.1**, **36.2**, **37.1**, **38.1**, and **39.1** were characterized for the presence in the MS^2 spectra of an intense signal at *m/z* 285, which is diagnostic of the kaempferol aglycone (Fabre et al., 2001). Based on the same rules, as reported above for quercetin, these compounds were assigned to kaempferol-derivatives, as depicted in **Table 1**. Similarly, compounds **27.4** and **30.2** were characterized for the presence of the diagnostic peaks of the myricetin aglycone (*m/z* 317 and 179) in the MS^2 spectra and identified as myricetin-derivatives as reported in **Table 1** (Calani et al., 2013). Finally, two isorhamnetin-derivatives (compounds **11.3** and **32.1**) were identified in the *E. hirta* leaves extracts (**Table 1**) (Mena et al., 2016).

3.1.4. Flavan-3-ols, flavones, dihydroflavonols and isocoumarins

Five flavan-3-ols were identified in the *E. hirta* leaves extracts (**Table 1**). Epicatechin (compound **20.2**; *m/z* 289) was identified by comparison of retention time and fragmentation spectra with the authentic standard. Three type-B procyanidin dimers ((epi)catechin-(epi)catechin) were identified at *m/z* 577 (compounds **14.3**, **17.3** and **20.3**). The fragmentation pattern reported in **Table 1** is consistent with previously reported data (Gu et al., 2003). Compound **27.6** showed a pseudomolecular ion at *m/z* 597 and MS^2 fragments at *m/z* 435 and 315. The fragment at m/z 435 revealed the loss of *O*-linked hexoside group whereas the subsequent loss of 120 Da (fragment at *m/z* 315) is characteristic of a *C*-linked hexoside group. Fragmentation did not generate the aglycone, but it can be obtained through the calculation $[M-H]^-$-162-120-42 (Waridel et al., 2001). The compound was tentatively identified as (epi)afzelechin-*O*-hexoside-*C*-hexoside.

Five flavones were identified in the *E. hirta* leaves extracts (**Table 1**). Compound **42.1** was assigned to the aglycone chrysin based on previously published fragmentation pathway (Fabre et al., 2001). Compounds **22.1**, **24.1**, **25.5** and **30.3** were instead identified as apigenin-derivatives. Compound **22.1** presented a pseudomolecular ion at *m/z* 415 with a single peak in the MS^2 spectra at *m/z* 269 originating from the loss of a rhamnosyl moiety and corresponding to the aglycone of apigenin (Fabre et al., 2001). Compound **30.3** (*m/z* 431) was identified as apigenin-6-*C*-glucoside due to the presence of the peak at *m/z* 341 (-90 Da) and 311 (-120 Da) diagnostic for a *C*-linked hexoside group, and at *m/z* 413 (-18 Da) diagnostic of a 6-*C*-glycosidic bond (Waridel et al., 2001). Compound **25.5** showed a negative molecular ion at *m/z* 563 and fragment ions at *m/z* 473, 443 and 413 resulting from the loss of 90, 120 and 150 Da, respectively, indicating the linkage of hexoside to the *C*-position of aglycone (Ferreres et al., 2007). The presence of the fragment at m/z 545 (-18 Da) is diagnostic of a 6-*C*-hexoside bond. The fragments at *m/z* 383 and 353 are instead indicative of the presence of an 8-*C*-linked pentoside moiety (Waridel et al., 2001). The compound was identified as apigenin-6-*C*-hexoside-8-*C*-pentoside. Compound **24.1** (*m/z* 593) generated in the MS^2 spectra fragment at *m/z* 473 (loss of 120 Da), 431 (loss of 162 Da from the parent ion) and 311 (loss of 120 Da from the ion at *m/z* 431). This compound was therefore identified as apigenin-8-*C*-hexoside-4'-*O*-hexoside. Three dihydroflavonols were identified in the mass spectrum. Compound **24.2** showed a pseudomolecular ion at *m/z* 449 and was identified as dihydrokaempferol-7-*O*-hexoside due to the presence of the signals at *m/z* 287 (dihydrokaempferol aglycone originating from the loss of the hexoside moiety) and 259 (characteristic of 7-*O*-glycosyl linkage). Compounds **16.2** and **19.3** showed the same negative molecular ion (*m/z* 465) and a fragmentation pattern typical of taxifolin-hexoside (Martini et al., 2017). Finally, 5 isocoumarins (brevifolin-derivatives), corresponding to compounds **3.2**, **7.2**, **17.4**, **21.1** and **23.1**, were found in the extract (**Table 1**). They were characterized for the presence of peaks in the MS^2 spectra corresponding to the brevifolin aglycone (*m/z* 247) and brevifolin-carboxylic acid (*m/z* 291) (Lantzouraki et al., 2015).

313

314 *3.1.5. Hydroxycinnamic acids*

315 Compounds **1.1**, **25.1** and **34.1** were easily identified by comparison with authentic standards. On
316 the other hand, compounds **2.1**, **6.4**, **11.4**, **12.1**, **19.1** and **23.5** (*m/z* 353) were identified as
317 caffeoylquinic acids (CQAs) using the hierarchical keys previously developed by Clifford et al.
318 (2003) and the order of elution (Martini et al., 2017). Indeed, two isomers of 5-*O*-coumaroylquinic
319 acid (compounds **10.1** and **25.2**; *m/z* 337) and one of 5-*O*-feruloylquinic acid (compound **27.5**; *m/z*
320 367) were identified using the same hierarchical keys as reported above (Clifford et al., 2003;
321 Martini et al., 2017). Compound **34.2** showed a negative molecular ion at *m/z* 309 and a product ion
322 at *m/z* 193 (ferulic acid aglycone) due to the loss of a malic acid residue (-116 Da). Compound **14.1**
323 (*m/z* 341) was identified as caffeic acid-*O*-hexoside due to the presence of the peaks at *m/z* 179 (loss
324 of hexose residue) and 135 which are characteristic of caffeic acid (Martini et al., 2017). Compound
325 **10.4** (m/z 515) showed a fragmentation pattern typical of 3,5-*O*-dicaffeoylquinic acid (Clifford et
326 al., 2005). Compound **5.3** showed a pseudomolecular ion at *m/z* 517 and fragment in the MS^2
327 spectra at *m/z* 337 (loss of 180 Da associated with a coniferyl alcohol moiety) and 193 (loss of
328 hexose). This compound was identified as feruloyl-coniferin (Mena et al., 2012). Finally,
329 compounds **14.4** and **19.4** (*m/z* 529) were assigned to feruloyl-caffeoylquinic acid (Clifford et al.,
330 2006).

331

332 *3.1.6. Hydroxybenzoic acids*

333 Compounds **6.1**, **13.1** and **26.1** were easily identified by comparison with authentic standards.
334 Compounds **4.2**, **5.2**, **6.2** and **8.1** with a parent ion $[M-H]^-$ at *m/z* 331 revealed a daughter ion
335 $[M-H-162]^-$ at *m/z* 169 upon fragmentation, indicating the loss of a hexosyl moiety. They were
336 identified as galloyl-*O*-hexoside isomers (Erşan et al., 2016). The parent ion $[M-H]^-$ at *m/z* 493 of
337 compound **5.4** formed daughter ions $[M-H-162]^-$ at *m/z* 313 and $[M-H-162-162]^-$ at *m/z* 169 and

was tentatively identified as a galloyl-di-*O*-hexoside. Compounds **4.1**, **5.5** and **6.6** (*m/z* 343) were identified as galloyl-quinic acid isomers due to the presence in the MS^2 spectra of peak at 191 (quinic acid moiety generated by the loss of a galloyl moiety) and 169 (galloyl moiety generated by the loss of a quinic acid moiety) (Erşan et al., 2016). Compounds **15.1** and **21.3** were tentatively identified as di- and tri-galloylquinic acids due to sequential losses of galloyl moieties (152 Da) from their parent ions at *m/z* 495 and 647, respectively, and the formation of a final product ion at *m/z* 191 (quinic acid moiety) (Erşan et al., 2016). Compound **27.1** presented a pseudomolecular ion at *m/z* 505, which generated the daughter ions at *m/z* 343 (galloylquinic acid moiety; due to the loss of a hexose group) and 313 (gallic acid hexose moiety; due to the loss of a quinic acid moiety). This compound was identified as galloylquinic acid-*O*-hexoside. Compounds **108** and **119**, exhibited parent ions $[M-H]^-$ at *m/z* 321 and 473. Their fragmentations resulted in product ions at *m/z* 169 and 125 characteristic of gallic acid. Thus, these compounds were tentatively identified as di- and tri-gallic acids, due to sequential loss of galloyl moieties, yielding product ions specific for gallic acid. Three additional compounds (**106**, **109** and **117**) showed the presence in the MS^2 spectra of the typical product ions of gallic acid (*m/z* 169 and 125). Compound **106** (*m/z* 197) was characterized for a loss of 48 Da generated by ethylic group. This compound was tentatively identified as ethyl-gallic acid. Compound **109** exhibited a pseudomolecular ion $[M-H]^-$ at *m/z* 325 and was characterized by the loss of 156 Da, yielding a daughter ion at m/z 169. This compound was identified as galloyl-shikimic acid (Erşan et al., 2016). Compound **117** (*m/z* 437) generated after fragmentation a peak at *m/z* 169 and was identified as galloyl-salicilin (Itoh et al., 2000). Compounds **107** and **118** showed negative parent ions at *m/z* 315 and 447, respectively, and their fragmentations resulted in product ions at *m/z* 153 and 109 characteristic of protocatechuic acid. For the compound **107** the signal at m/z 153 resulted from the loss of a hexose group and was identified as protocatechuic acid-*O*-hexoside (Martini et al., 2017). Compound **118** presented in the MS^2 spectra a fragment at 315 (protocatechuic acid-hexoside group) arising from the loss of a pentose

group (-132 Da). This compound was tentatively identified as protocatechuic acid-*O*-hexoside-*O*-pentoside.

3.1.7. Other phytochemicals

Seven organic acids (compounds **a.1**, **a.2**, **b.1**, **c.1**, **d.1**, **f.1** and **p.4**, **Table 2**) were easily identified in the *E. hirta* extracts due to the characteristic fragmentation patterns that resulted in the loss of H_2O (-18 Da) and/or CO_2 (-44 Da) (Brent et al., 2014). Two *ent*-kaurene diterpenoids, albopilosin H (*m/z* 331; compound **i.1**) and ponicidin (*m/z* 361; compound **l.1**) were identified according to the fragmentation scheme proposed by Zhou et al. (2008). Based on the same scheme an *ent*-6,7-*seco*-diterpenoids, isojaponins A (*m/z* 377; compound **l.2**), was identified in the extract (Zhou et al., 2009). An additional diterpenoid, gibberellin CA29 (*m/z* 347; compound **e.1**), was identified basing on the fragmentation spectrum reported by Urbanová et al. (2013). Two additional signals in the negative mass spectra were assigned to crysophanol-8'-O-(6'-O-galloyl)-glucose (*m/z* 567; compound **h.1**) and roseoside (*m/z* 385; compound **g.1**) (Cádiz-Gurrea et al., 2013; Ye et al., 2007). In the positive MS spectra, 5 additional signals were identified. Three of them belonged to the aromatic amino acids phenylalanine (*m/z* 166; compound **p.1**), tyrosine (*m/z* 182; compound **p.2**) and tryptophan (*m/z* 205; compound **p.3**). Compound **p.5** was instead identified as the dipeptide glutamic acid-tyrosine *(m/z* 311). Finally, the last signal (*m/z* 466; compound **p.6**) was assigned to the alkaloid ternatoside C (Zhang et al., 2007).

3.2. Quantitative profile of phenolic compounds in the Euphorbia hirta leaves

Tables 3-6 and **Figure 2** provide information about the amount of the 123 tentatively identified phenolic compounds in the water and ethanol extracts of *E. hirta* leaves.

Water extract of *E. hirta* leaves contained more phenolic compounds than the ethanol extract, 163.62 ± 0.61 mg/g of extract vs 49.61 ± 0.39 mg/g of extract ($P< 0.05$), respectively. Water extract

388 was particularly rich in gallotannins and hydroxybenzoic acids (representing the 31.4% and 26.5%
389 of total phenolic compounds, respectively) (**Tables 3** and **6** and **Figure 2A**), whereas the ethanol
390 extract was rich in hydroxycinnamic acids and isocoumarin (representing the 45% and 16.7% of
391 total phenolic compounds, respectively) (**Tables 5** and **6** and **Figure 2B**). In the ethanol extract,
392 feruloyl-coniferin represented alone the 31.7% of total phenolic compounds and the 70.3% of total
393 hydroxycinnamic acids (**Table 6**).
394 **Figure 3** details the structure of the most important phenolic compounds identified in the *E. hirta*
395 leaves.
396
397 *3.3. Antioxidant activity analysis*
398 To fully characterize the antioxidant properties of the two extracts, the ability to scavenge
399 physiologically relevant radicals (superoxide anions), the organic nitro-radical ABTS and the
400 reducing power were evaluated. In addition, the Fe^{2+}-chelating ability of the two extracts was
401 assessed. The ethanol extract of *E. hirta* leaves was more effective, with respect to the
402 corresponding water extract, in scavenging ABTS ($P< 0.05$) and superoxide anion radicals ($P<$
403 0.05), despite the lower phenolic content measured by LC-MS analysis (**Figure 4**). Furthermore, the
404 ethanol extracts also showed higher reducing power with respect to the water extract ($P< 0.05$).
405 These results may be due the presence of non-phenolic antioxidant compounds or of unidentified
406 phenolic compounds in the ethanol extract. Alternatively, phenolic compounds present in the
407 ethanol extract may have a better antioxidant potential than those in the water extract. On the other
408 hand, the water extract exhibited better chelating ability towards Fe^{2+} than the ethanol extract ($P<$
409 0.05).
410
411 *3.4. Antifungal activity analysis*

412 The *in vitro* antifungal activity of *E. hirta* leaves extracts was assayed, in order to check their
413 activity in inhibiting fungal growth. The assessment of any antifungal activity is pivotal for the
414 development and implementation of a suitable technology for the production of novel bio-
415 fungicides based on the exploitation of a possible antifungal activity of such extracts.
416 The extracts of *E. hirta* leaves displayed higher effectiveness in reducing the myceliar growth of
417 three pathogenic fungi to tomato, *R. solani*, *F. oxysporum* f. sp. *vasinfectum*, and *A. solani*, in a
418 concentration-dependent manner (**Table 7**). The ethanol extract was more effective in inhibiting
419 fungal growth than the water extract ($P < 0.05$; **Figure S2**). These results are in agreement with
420 those of other authors reporting that the *in vitro* antifungal and antimicrobial activities of some
421 ethanol extracts had higher efficacy than the aqueous extracts (Eloff, 1998; Kotze and Eloff, 2002;
422 Dakole et al., 2016).
423 Several plant extracts have been tested for their antifungal activity against the three pathogenic
424 fungi analysed in this study. Methanolic extracts of leaves from *Pulicaria incisa*, *Rhanterium*
425 *epapposum* and *Horwoodia dicksoniae* showed higher antifungal activity against *F. oxysporum* than
426 the *E. hirta* leaves extracts (Mohamed et al., 2017). However, *E. hirta* ethanol extract was as
427 effective as *Citrullus colocynthis* and *Gypsophila capillaris* leaves methanolic extracts (Mohamed
428 et al., 2017). Indeed, *E. hirta* ethanol extract displayed higher antifungal activity against *F.*
429 *oxysporum* and *A. solani* than *Vitis vinifera, Punica granatum* and *Ficus carica* leaves methanolic
430 extracts (El-Khateeb et al., 2013). The aqueous extracts of *Polystichum squarrosum, Adiantum*
431 *venustum Parthenium hysterophorus, Urtica dioeca* and *Cannabis sativa* leaves exhibited
432 antifungal activity against *R. solani*, *F. oxysporum* and *A. solani* with a lower effectiveness respect
433 to *E. hirta* ethanol and water extracts (Tapwall et al., 2011). Rongai et al. (2015) investigated the
434 antifungal properties of aqueous extracts from twenty plants against *F. oxysporum*. Among them,
435 extracts of *Rivina humulis, Brassica carinata, Brunfelsia calyicina, Salvia guaranitica* and *Punica*

436 *granatum* showed the best antifungal activity. Nevertheless, they were less effective than the *E.*
437 *hirta* water extract tested in this study.
438 The linearly increasing efficacy related to the concentration is a clear indication of the presence of
439 antifungal molecules in both extracts. Despite the higher amount of phenolic compounds identified
440 in water extract, a more pronounced antifungal activity was obtained using the ethanol extract ($P<$
441 0.05). This might be related to the very high content in isocoumarins and hydroxycinnamic acids of
442 the latter. Isocoumarins and hydroxycinnamic acids are well known phenolics, able to confer and/or
443 induce a non-specific resistance to several phytopathogens, when they affect their host plants. Such
444 plant-derived molecules belong to a group of antimicrobial substances called phytoalexins (Ingham,
445 1972) and are secondary metabolites produced in plants, especially as a result of biotic stresses
446 (Hammerschmidt, 1999).
447 Some of the phenolic compounds detected in the ethanol extract in higher amount, with respect to
448 the water extract, are described to possess a marked antifungal activity. For instance, ferulic and
449 coumaric acids showed a remarkable *in vitro* inhibiting effect on the growths of *F. oxysporum* and
450 *R. solani* (El Modafar and El Boustani, 2001; Hayashi, 1997). These compounds were also found in
451 higher concentration in date palm cultivars resistant to *F. oxysporum* infection, when compared to
452 the susceptible cultivars (El Modafar and El Boustani, 2001). Gallic acid showed antifungal activity
453 against *F. oxysporum* and *A. solani* (Alves Breda et al., 2016; Wu et al., 2009). Feruloyl-coniferin,
454 which represented alone more than 30% of the phenolic compounds in *E. hirta* leaves ethanol
455 extract (**Table 6**), seems to be particularly interesting. It is an ester between a molecule of coniferin
456 and a ferulic acid moiety (**Figure 3**). As reported above, ferulic acid was a potent inhibitor of fungal
457 growth, whereas coniferin was able to inhibit *in vitro* the growth of the pathogenic fungus
458 *Verticillium longisporum* (König et al., 2014). Indeed, mutant *Arabidopsis thaliana* plant lines
459 producing a high amount of coniferin were particularly resistant to *Verticillium longisporum*
460 infection (König et al., 2014). Induction of ferulic and coumaric acids synthesis is a common plant

461 defence mechanism to fungal infections. Panina et al. (2007) reported protection of tomato from *F.*
462 *oxysporum* as a consequence of ferulic and coumaric acids synthesis induced by the biocontrol non-
463 pathogenic fungus *F. oxysporum* CS-20 strain. Similarly, the biocontrol fungus *Pythium*
464 *oligandrum* elicited the accumulation of ferulic acid, protecting wheat from *Fusarium germinatum*
465 (Takenaka et al., 2003). Increased ferulic and coumaric acids level has been also associated to
466 tomato resistance to pathogens in resistant cultivars (Gayoso et al., 2010). The exact antifungal
467 mechanism of phenolic compounds is not yet fully elucidated, but may involve direct fungilytic
468 activity by disrupting cell membrane as well as inhibition of mycelial growth or the activation of
469 specific signalling pathways (Hayashi, 1997; Martins et al., 2015; Shalaby et al., 2016).
470
471 **4. Conclusion**
472 From this study, it emerges that *E. hirta* L. might be a potential and very rich source of phenolic
473 classes, such as gallotannins, hydroxybenzoic and hydroxycinnamic acids, and bioactive
474 components especially tri-*O*-galloyl-glucose isomers, feruloyl-coniferin, tetra-*O*-galloyl-glucose
475 isomers, di-*O*-galloyl-glucose isomers, ethyl-gallic acid, protocatechuic acid-*O*-pentoside-*O*-
476 hexoside, 5-*O*-caffeoyl-quinic acid *trans* isomer and digalloyl-quinic acid. The development and
477 implementation of new fungicides from these phenolics or, alternatively, the use of purified extracts
478 from *E. hirta*, may provide a new approach to control fungal diseases in tropical areas where, often,
479 sustainability of chemical control measures are not met. Additionally, since *E. hirta* is a very
480 common weed, the use of its extracts may provide an additional income to rural areas.

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Figure captions

Fig. 1. Representative negative ion mode base peak chromatograms (BPCs) of water (A) and ethanol (B) extracts from *Euphorbia hirta* leaves. The shown BPCs are representative of three independent experiments**.**

Fig. 2. Occurrence of phenolic classes in *Euphorbia hirta* extracts. Global percentage of flavan-3-ols, flavonols, di-hydro-flavonols, hydroxybenzoic and hydroxycinnamic acids, gallotannins, ellagitannins, flavones and isocoumarins in water (A) and ethanol (B) extracts of *Euphorbia hirta* leaves. The total amounts of phenolic compounds quantified with mass spectrometry is also shown.

Fig. 3. Structures of some newly identified *Euphorbia hirta* leaves phenolic compounds. Examples of the phenolic structures present in highest concentration in the *Euphorbia hirta* leaves. (A) R^1: -H, coumaric acid; $-CH_3$, ferulic acid; (B) R^1: -OH, caffeoyl-quinic acid; $-CH_3$, feruloyl-quinic acid; (C) feruloyl-coniferin; (D) quercetin-7-*O*-pentoside; (E) apigenin-6-*C*-hexoside; (F) kaempferol-3-*O*-hexoside; (G) R^1: -OH, gallic acid; $-CH_2CH_3$, ethyl-gallic acid; (H) di-galloyl-quinic acid; (I) tri-galloyl-quinic acid; (J) gallotannins (R^1-R^5 may be –OH or –gallic acid); (K) brevifolin-carboxylic acid; (L) protocatechuic acid-*O*-pentoside-*O*-hexoside (R^1 and R^2 may identified a pentoside or hexoside moiety).

Fig. 4. Antioxidant properties of water (black columns) and ethanol (grey columns) extracts from *Euphorbia hirta* leaves. Antioxidant capacity (expressed as μmol ascorbic acid/g of powder) measured by three different assays (left *y*-axys). SOA: superoxide anion scavenging activity. The right *y*-axys detailed the Fe^{2+}-chelating ability of the two extracts expressed as percentage of bound Fe^{2+}. Each sample was run in triplicate and results are reported as mean values ± SD. Values with different letter within the same assay are significantly different ($P < 0.05$).

Table 1. Mass spectra data for phenolic compounds identified in water and ethanol extracts from *Euphorbia hirta* leaves.

Peak		Compound	$[M-H]^-$ *(m/z)*	MS^2 ion fragments *(m/z)*	Water extract	Ethanol extract
1	1.1	Coumaric acid	163	119, 101	-	+
2	2.1	4-*O*-caffeoyl-quinic acid *cis*	353	173, 191	+	-
	2.2	HHDP-hexoside isomer	481	301, 275	+	-
3	3.1	HHDP-hexoside isomer	481	301, 275	+	-
	3.2	Dihydro-hydroxy-brevifolin-dicarboxylic acid	353	291, 247, 203, 335	-	+
4	4.1	Galloyl-quinic acid isomer	343	191, 169, 125	+	+
	4.2	Galloyl-glucose isomer	331	169, 125, 271, 211	+	+
5	5.1	HHDP-hexoside isomer	481	301, 275	+	-
	5.2	Galloyl-glucose isomer	331	169, 125, 271, 211	+	-
	5.3	Feruloyl-coniferin	517	337, 193, 175, 217	+	+
	5.4	Galloyl-di-*O*-hexoside	493	331, 313, 271, 169, 211	+	-
	5.5	Galloyl-quinic acid isomer	343	191, 169, 125	+	-
6	6.1	Gallic acid	169	125	+	+
	6.2	Galloyl-glucose isomer	331	169, 125, 271, 211	+	-
	6.3	Ellagitannin	847	481, 301	+	-
	6.4	Gallotannin	465	271, 169, 313, 301	+	+
	6.5	4-*O*-caffeoyl-quinic acid *trans*	353	173, 191	+	-
	6.6	Galloyl-quinic acid isomer	343	191, 169, 125	+	-
	6.7	Galloyl-HHDP-hexoside isomer (corilagin)	633	301, 481, 275	+	-
7	7.1	Ellagitannin	681	481, 301, 663, 619	-	+
	7.2	Brevifolin-dicarboxylic acid-hexoside isomer	497	335, 291, 247, 203	-	+
8	8.1	Galloyl-glucose isomer	331	169, 125, 271, 211	+	-
9	9.1	Ellagitannin	681	481, 301, 663, 619	-	+
10	10.1	5-*O*-coumaroyl-quinic acid trans	337	191, 173, 233, 337	+	+
	10.2	Galloyl-shikimic acid	325	169, 125	+	-
	10.3	Di-*O*-galloyl-glucose isomer	483	271, 193, 211, 169, 313, 331	+	-
	10.4	3,5-*O*-dicaffeoyl-quinic acid	515	191, 353, 179, 173	+	-
	10.5	Protocatechuic acid-*O*-hexoside	315	153, 109	+	-

11	11.1	Galloyl-HHDP-hexoside isomer (corilagin)	633	301, 481, 275	+	+
	11.2	Protocatechuic acid-*O*-pentoside-*O*-hexoside	447	315, 153	+	-
	11.3	Isorhamnetin-3-*O*-pentoside	447	315, 300, 301	+	-
	11.4	3-*O*-caffeoyl-quinic acid cis	353	191, 179, 135	+	+
12	12.1	3-*O*-caffeoyl-quinic acid trans	353	191, 179, 135	+	-
13	13.1	Protocatechuic acid	153	109	-	+
14	14.1	Caffeic acid-*O*-hexoside	341	179, 135	+	-
	14.2	Kaempferol-3-*O*-hexoside isomer	447	285, 255	+	-
	14.3	Procyanidin dimer B-type isomer	577	407, 289, 245, 425	+	-
	14.4	Feruloyl-caffeoyl-quinic acid isomer	529	353, 173	+	-
	14.5	Di-*O*-galloyl-glucose isomer	483	271, 193, 211, 169, 313, 331	+	-
	14.6	Quercetin-3-*O*-hexoside-7-*O*-hexoside	625	463, 301, 273, 271	+	-
15	15.1	Digalloyl-quinic acid	495	343, 191, 169	+	-
16	16.1	Tri-*O*-galloyl-glucose isomer	635	271, 193, 211, 483, 169, 313, 331	+	-
	16.2	Taxifolin-3-*O*-hexoside isomer	465	303, 285, 241	+	-
17	17.1	bis-HHDP-hexoside (pedunculagin I)	783	301, 275	-	+
	17.2	Dehydro-galloyl-HHDP-hexoside isomer	631	451, 301, 275	+	+
	17.3	Procyanidin dimer B-type isomer	577	407, 289, 245, 425	+	-
	17.4	Brevifolin-carboxylic acid-hexoside isomer	453	291, 247	+	+
	17.5	Digallic acid	321	277, 169, 125	-	+
18	18.1	Dehydro-galloyl-HHDP-hexoside isomer	631	451, 301, 275	+	+
19	19.1	5-*O*-caffeoyl-quinic acid trans	353	191	+	-
	19.2	Tri-*O*-galloyl-glucose isomer	635	271, 193, 211, 483, 169, 313, 331	+	-
	19.3	Taxifolin-3-*O*-hexoside isomer	465	303, 285, 241	+	-
	19.4	Feruloyl-caffeoyl-quinic acid isomer	529	353, 173	+	-
20	20.1	Galloyl-HHDP-hexoside isomer (corilagin)	633	301, 481, 275	+	+
	20.2	Epicatechin	289	245, 205, 179, 125	+	+
	20.3	Procyanidin dimer B-type isomer	577	407, 289, 245, 425	+	-
21	21.1	Brevifolin-carboxylic acid	291	247	+	+

	21.2	Granatin B isomer	951	933, 301	+	-
	21.3	Tri-galloyl-quinic acid	647	495, 343	-	+
22	22.1	Apigenin-7-*O*-rhamnoside	415	269	+	-
	22.2	Di-galloyl-HHDP-hexoside (pedunculagin II)	785	481, 301	+	-
23	23.1	Brevifolin-carboxylic acid-galloyl-hexoside	605	453, 291, 247	+	+
	23.2	Tri-*O*-galloyl-glucose isomer	635	271, 193, 211, 483, 169, 313, 331	+	-
	23.3	Quercetin-3-*O*-galactoside	463	301, 179, 271, 151	+	-
	23.4	Tetra-*O*-galloyl-glucose isomer	787	635, 617, 483, 301	+	-
	23.5	5-*O*-caffeoyl-quinic acid cis	353	191	+	-
24	24.1	Apigenin-8-*C*-hexoside-4'-*O*-hexoside	593	473, 431, 311, 301, 179, 271, 151	+	+
	24.2	Dihydro-kaempferol-7-*O*-hexoside	449	287, 269, 259	+	-
	24.3	Di-*O*-galloyl-rhamnose	467	423, 315, 169	+	-
	24.4	Kaempferol-7-*O*-hexoside-3-*O*-rutinoside	755	593, 375, 285, 255	+	-
25	25.1	Caffeic acid	179	135	+	+
	25.2	5-*O*-coumaroyl-quinic acid cis	337	191, 173, 233, 337	+	-
	25.3	Kaempferol-3-*O*-hexoside isomer	447	285, 255	+	-
	25.4	Galloyl-salicin	437	313, 169, 125	+	-
	25.5	Apigenin-6-*C*-hexoside-8-*C*-pentoside	563	545, 473, 443, 413, 383, 353, 303	+	+
26	26.1	Dihydroxy-benzoic acid	153	109	-	+
27	27.1	Galloyl-quinic acid-*O*-hexoside	505	343, 313, 169	+	-
	27.2	Granatin B isomer	951	933, 301	+	-
	27.3	Tetra-*O*-galloyl-glucose isomer	787	635, 617, 483, 301	+	-
	27.4	Myricetin-3-*O*-hexoside	479	433, 316, 287, 179	+	-
	27.5	5-*O*-feruloyl-quinic acid	367	191, 173	+	-
	27.6	(Epi)afzelechin-*C*-hexoside-*O*-hexoside	597	435, 315	+	-
28	28.1	Ellagic acid-malonyl-pentoside isomer	519	301, 501, 484, 413, 319, 275, 229, 199	-	+
29	29.1	Quercetin-3-*O*-rutinoside	609	301, 271, 179, 151	+	-
30	30.1	Penta-*O*-galloyl-glucose	939	785, 769, 617	+	-
	30.2	Myricetin-3-*O*-pentoside	449	316, 317, 287, 179	+	-

	30.3	Apigenin-6-*C*-hexoside	431	341, 311, 283, 413	+	+
	30.4	Ellagic acid-malonyl-pentoside isomer	519	301, 501, 484, 413, 319, 275, 229, 199	-	+
31	31.1	Quercetin-3-*O*-glucoside	463	301, 179, 271, 151	+	+
	31.2	Kaempferol-3-*O*-hexoside isomer	447	285, 255	+	-
	31.3	Ethyl-gallic acid	197	169, 125	+	+
	31.4	Ellagitannin	765	301, 463, 626, 229	+	-
	31.5	Ellagic acid	301	271, 229	+	+
	31.6	Quercetin-3-*O*-glucuronide	477	301, 179, 271, 151	+	+
	31.7	Quercetin-3-*O*-galloyl-hexoside isomer	615	463, 301, 271	+	-
32	32.1	Isorhamnetin-3-*O*-rutinoside	623	315, 300, 301, 179	+	-
	32.2	Kaempferol-3-*O*-rutinoside	593	285, 255	+	-
	32.3	Quercetin-3-*O*-pentoside	433	301, 271, 179, 151, 300	+	-
	32.4	Quercetin-3-*O*-acetyl-hexoside isomer	505	300, 301, 463, 271, 179, 151	+	-
	32.5	Gallagic acid	601	313, 287, 211, 169	+	-
33	33.1	Gallotannin	659	465, 313, 489	+	-
	33.2	Kaempferol-3-*O*-hexoside isomer	447	285, 255	+	-
	33.3	Quercetin-7-*O*-pentoside	433	301, 273, 179, 151, 300	+	-
	33.4	Quercetin-3-*O*-rhamnoside	447	301, 179, 151, 271	+	-
	33.5	Quercetin-7-*O*-galloyl-pentoside isomer	585	433, 301, 273, 179, 255	+	-
	33.6	Kaempferol-3-*O*-glucuronide	461	285, 255	+	-
34	34.1	Ferulic acid	193	178, 149, 134	+	+
	34.2	Feruloyl-malic acid	309	193	+	-
35	35.1	Quercetin-3-*O*-acetyl-hexoside isomer	505	300, 301, 463, 271, 179, 151	+	-
	35.2	Trigallic-acid	473	271, 211, 169	+	-
	35.3	Kaempferol-3-*O*-pentoside isomer	417	285, 284, 255	+	-
	35.4	Kaempferol-3-*O*-acetyl-hexoside	489	327, 285, 255	+	-
	35.5	Quercetin-7-*O*-galloyl-pentoside isomer	585	433, 301, 273, 179, 255	+	-
36	36.1	Kaempferol-3-*O*-pentoside isomer	417	285, 284, 255	+	-
	36.2	Kaempferol-3-*O*-rhamnoside	431	285, 255	+	-
37	37.1	Kaempferol-7-*O*-galloyl-pentoside isomer	569	285, 257, 417	+	-

	37.2	Quercetin-3-*O*-galloyl-hexoside isomer	615	463, 301, 271	+	-
	37.3	Quercetin-7-*O*-galloyl-pentoside isomer	585	433, 301, 273, 179, 255	+	-
38	38.1	Kaempferol-7-*O*-galloyl-pentoside isomer	569	285, 257, 417	+	-
39	39.1	Kaempferol-7-*O*-galloyl-pentoside isomer	569	285, 257, 417	+	-
40	40.1	Quercetin	301	151, 179	+	-
41	41.1	Deoxyellagic acid	285	257, 229, 185	+	-
42	42.1	Chrysin	253	209	+	-

HHDP: 2,3-(S)-hexahydroxydiphenoyl

Table 2. Mass spectra data for non-phenolic phytochemicals identified in water and ethanol extracts from *Euphorbia hirta* leaves.

Peak	Compound		[M-H]$^-$ *(m/z)*	MS^2 ion fragments *(m/z)*	Sample[a]	Class
a	a.1	Hydroxycitric acid	207	163, 119, 101	EE	Organic acid
	a.2	Quinic acid	191	111	WE and EE	Organic acid
b	b.1	Shikimic acid	173	155, 111	EE	Organic acid
c	c.1	Citric acid	191	173, 111	WE and EE	Organic acid
d	d.1	Malic acid	133	115	EE	Organic acid
e	e.1	Gibberelin CA29	347	303, 259, 163, 150	EE	Terpene
f	f.1	Chelidonic acid	183	139	EE	Organic acid
g	g.1	Roseoside	385	223, 153	WE	Norisoprenoid
h	h.1	Chrysophanol-8'-O-(6'-O-galloyl)-glucose	567	331, 313, 271, 211, 169	WE	Anthraquinone
i	i.1	Albopilosin H	331	313, 295, 255, 241	EE	Terpene
L	l.1	Ponicidin	361	343, 325, 315, 271, 253, 235	EE	Terpene
	l.2	Isojaponins A	377	359, 341, 315, 297	EE	Terpene
p	p.1	Phenylalanine[b]	166	120	WE and EE	Amino acid
	p.2	Tyrosine[b]	182	165, 136	WE and EE	Amino acid
	p.3	Tryptophan[b]	205	188, 159, 144	EE	Amino acid
	p.4	Gluconic acid[b]	235	118	WE and EE	Organic acid
	p.5	Glutamic acid-tyrosine[b]	311	182, 165, 136	WE and EE	Dipeptide
	p.6	Ternatoside C[b]	466	304, 258, 190	EE	Alkaloid

[a]WE means that the compound was found in the aqueous extract whereas EE in the ethanol extract
[b]Indicates $[M+H]^+$ rather than $[M-H]^-$

Table 3. Quantitative data for tannins (ellagitannins and gallotannins) identified in water and ethanol extracts from *Euphorbia hirta* leaves. Values are expressed as mg/g of dry extract and represent means ± standard deviation of triplicate determination (n.d. means not detected).

	Compound	Water extract (mg/g)	Ethanol extract (mg/g)
Ellagitannins[a]			
41.1	Deoxyellagic acid	0.80 ± 0.01	n.d.
31.5	Ellagic acid	1.40 ± 0.03	0.40 ± 0.01
2.2	HHDP-hexoside isomer	0.13 ± 0.01	n.d.
3.1	HHDP-hexoside isomer	0.21 ± 0.01	0.42 ± 0.01
5.1	HHDP-hexoside isomer	0.25 ± 0.01	n.d.
28.1	Ellagic acid-malonyl-pentoside isomer	n.d.	0.59 ± 0.02
30.4	Ellagic acid-malonyl-pentoside isomer	n.d.	0.54 ± 0.01
32.5	Gallagic acid	0.64 ± 0.03	n.d.
17.2	Dehydro-galloyl-HHDP-hexoside isomer	0.25 ± 0.01	0.24 ± 0.01
18.1	Dehydro-galloyl-HHDP-hexoside isomer	0.28 ± 0.01	0.17 ± 0.01
6.7	Galloyl-HHDP-hexoside (corilagin) isomer	0.14 ± 0.02	n.d.
11.1	Galloyl-HHDP-hexoside (corilagin) isomer	1.22 ± 0.09	0.26 ± 0.01
20.1	Galloyl-HHDP-hexoside (corilagin) isomer	1.95 ± 0.01	0.29 ± 0.02
6.3	Ellagitannin	n.d.	0.19± 0.01
7.1	Ellagitannin	n.d.	0.26 ± 0.01
9.1	Ellagitannin	0.27 ± 0.01	n.d.
17.1	bis-HHDP-hexoside (pedunculagin I)	n.d.	0.35 ± 0.02
22.2	Di-galloyl-HHDP-hexoside (pedunculagin II)	0.23 ± 0.01	n.d.
31.4	Ellagitannin	0.01 ± 0.00	n.d.
21.2	Granatin B isomer	0.88 ± 0.01	n.d.
27.2	Granatin B isomer	0.68 ± 0.01	n.d.
	Total ellagitannins	**9.32 ± 0.10 (5.7%)**	**3.52 ± 0.03 (7.1%)**

Gallotannins[b]			
6.4	Gallotannin	0.57 ± 0.04	0.45 ± 0.01
24.3	Di-*O*-galloyl-rhamnose	3.23 ± 0.02	n.d.
10.3	Di-*O*-galloyl-glucose isomer	4.10 ± 0.08	n.d.
14.5	Di-*O*-galloyl-glucose isomer	6.76 ± 0.11	n.d.
16.1	Tri-*O*-galloyl-glucose isomer	3.17 ± 0.04	n.d.
19.2	Tri-*O*-galloyl-glucose isomer	5.57 ± 0.03	n.d.
23.2	Tri-*O*-galloyl-glucose isomer	10.11 ± 0.34	n.d.
33.1	Gallotannin	0.85 ± 0.01	n.d.
23.4	Tetra-*O*-galloyl-glucose isomer	0.95 ± 0.09	n.d.
27.3	Tetra-*O*-galloyl-glucose isomer	14.35 ± 0.06	n.d.
30.1	Penta-*O*-galloyl-glucose	1.64 ± 0.01	n.d.
	Total gallotannins	**51.30 ± 0.39 (31.4%)**	**0.45 ± 0.01 (0.9%)**

[a]Quantified as ellagic acid equivalent

[b]Quantified as gallic acid equivalent

Water and ethanol extracts were prepared by dissolving 20 mg of powder obtained from the extraction procedures in 1 mL of the respective solvent.

HHDP: 2,3-(S)-hexahydroxydiphenoyl

Table 4. Quantitative data for flavonols identified in water and ethanol extracts from *Euphorbia hirta* leaves. Values are expressed as mg/g of dry extract and represent means ± standard deviation of triplicate determination (n.d. means not detected).

	Compound	Water extract (mg/g)	Ethanol extract (mg/g)
Flavonols[a]			
40.1	Quercetin	0.78 ± 0.01	n.d.
32.3	Quercetin-3-*O*-pentoside	1.57 ± 0.21	n.d.
33.3	Quercetin-7-*O*-pentoside	3.77 ± 0.20	n.d.
33.4	Quercetin-3-*O*-rhamnoside	0.83 ± 0.03	n.d.
23.3	Quercetin-3-*O*-galactoside	0.20 ± 0.01	n.d.
31.1	Quercetin-3-*O*-glucoside	1.96 ± 0.01	0.07 ± 0.00
31.6	Quercetin-3-*O*-glucuronide	0.48 ± 0.01	0.11 ± 0.00
32.4	Quercetin-3-*O*-acetyl-hexoside isomer	0.53 ± 0.01	n.d.
35.1	Quercetin-3-*O*-acetyl-hexoside isomer	0.09 ± 0.00	n.d.
33.5	Quercetin-7-*O*-galloyl-pentoside isomer	0.33 ± 0.02	n.d.
35.5	Quercetin-7-*O*-galloyl-pentoside isomer	0.39 ± 0.01	n.d.
37.3	Quercetin-7-*O*-galloyl-pentoside isomer	1.05 ± 0.02	n.d.
29.1	Quercetin-3-*O*-rutinoside	0.31 ± 0.01	n.d.
31.7	Quercetin-3-*O*-galloyl-hexoside isomer	0.07 ± 0.00	n.d.
37.2	Quercetin-3-*O*-galloyl-hexoside isomer	0.16 ± 0.01	n.d.
14.6	Quercetin-3-*O*-hexoside-7-*O*-hexoside	0.28 ± 0.01	n.d.
35.3	Kaempferol-3-*O*-pentoside isomer	1.03 ± 0.04	n.d.
36.1	Kaempferol-3-*O*-pentoside isomer	3.00 ± 0.10	n.d.
36.2	Kaempferol-3-*O*-rhamnoside	0.15 ± 0.02	n.d.
14.2	Kaempferol-3-*O*-hexoside isomer	0.15 ± 0.01	n.d.
25.3	Kaempferol-3-*O*-hexoside isomer	0.14 ± 0.01	n.d.

31.2	Kaempferol-3-*O*-hexoside isomer	0.12 ± 0.01	n.d.
33.2	Kaempferol-3-*O*-hexoside isomer	0.14 ± 0.01	n.d.
33.6	Kaempferol-3-*O*-glucuronide	0.17 ± 0.01	0.07 ± 0.00
35.4	Kaempferol-3-*O*-acetyl-hexoside	0.19 ± 0.01	n.d.
37.1	Kaempferol-7-*O*-galloyl-pentoside isomer	0.13 ± 0.01	n.d.
38.1	Kaempferol-7-*O*-galloyl-pentoside isomer	0.23 ± 0.01	n.d.
39.1	Kaempferol-7-*O*-galloyl-pentoside isomer	0.47 ± 0.02	n.d.
32.2	Kaempferol-3-*O*-rutinoside	0.59 ± 0.01	n.d.
24.4	Kaempferol-7-*O*-hexoside-3-*O*-rutinoside	0.12 ± 0.00	n.d.
30.2	Myricetin-3-*O*-pentoside	0.31 ± 0.01	n.d.
27.4	Myricetin-3-*O*-hexoside	0.16 ± 0.01	n.d.
11.3	Isorhamnetin-3-*O*-pentoside	0.72 ± 0.02	n.d.
32.1	Isorhamnetin-3-*O*-rutinoside	0.16 ± 0.01	n.d.
	Total flavonols	**20.78 ± 0.31 (12.7%)**	**0.26 ± 0.00 (0.5%)**

[a]Quantified as quercetin-3 glucoside equivalent with the exception of the kaempferol-derivative which were quantified as kaempferol equivalent

Water and ethanol extracts were prepared by dissolving 20 mg of powder obtained from the extraction procedures in 1 mL of the respective solvent.

Table 5. Quantitative data for flavan-3-ols, flavones, dihydroflavonols and isocoumarins identified in water and ethanol extracts from *Euphorbia hirta* leaves. Values are expressed as mg/g of dry extract and represent means ± standard deviation of triplicate determination (n.d. means not detected).

	Compound	Water extract (mg/g)	Ethanol extract (mg/g)
Flavan-3-ols[a]			
20.2	Epicatechin	0.39 ± 0.01	0.08 ± 0.01
14.3	Procyanidin dimer B-type isomer	0.10 ± 0.00	n.d.
17.3	Procyanidin dimer B-type isomer	0.80 ± 0.01	n.d.
20.3	Procyanidin dimer B-type isomer	0.23 ± 0.01	n.d.
27.6	(Epi)afzelechin-*C*-hexoside-*O*-hexoside	0.46 ± 0.02	n.d.
	Total flavan-3-ols	**1.97 ± 0.02 (1.2%)**	**0.08 ± 0.01 (0.2%)**
Flavones[b]			
42.1	Chrysin	0.16 ± 0.01	0.26 ± 0.01
22.1	Apigenin-7-*O*-rhamnoside	0.96 ± 0.01	n.d.
30.3	Apigenin-6-*C*-hexoside	2.18 ± 0.11	0.32 ± 0.01
25.5	Apigenin-6-*C*-hexoside-8-*C*-pentoside	0.33 ± 0.01	0.18 ± 0.01
24.1	Apigenin-8-*C*-hexoside-4'-*O*-hexoside	0.13 ± 0.01	0.12 ± 0.01
	Total flavones	**3.76 ± 0.11 (2.3%)**	**0.89 ± 0.01 (1.8%)**
Dihydroflavonols[b]			
24.2	Dihydro-kaempferol-7-*O*-hexoside	0.04 ± 0.00	n.d.
16.2	Taxifolin-3-*O*-hexoside isomer	0.17 ± 0.01	n.d.
19.3	Taxifolin-3-*O*-hexoside isomer	0.09 ± 0.00	n.d.
	Total dihydroflavonols	**0.30 ± 0.01 (0.2%)**	**n.d.**
Isocoumarins[c]			
21.1	Brevifolin-carboxylic acid	5.25 ± 0.13	3.61 ± 0.05

3.2	Dihydro-hydroxy-brevifolin-dicarboxylic acid	n.d.	4.86 ± 0.05
17.4	Brevifolin-carboxylic acid-hexoside	2.04 ± 0.10	2.73 ± 0.06
7.2	Brevifolin-dicarboxylic acid-hexoside	n.d.	2.09 ± 0.02
23.1	Brevifolin-carboxylic acid-galloyl-hexoside	2.27 ± 0.04	0.51 ± 0.01
	Total isocoumarins	**9.56 ± 0.17 (5.8%)**	**13.81 ± 0.09 (27.8%)**

[a]Quantified as catechin equivalent

[b]Quantified as quercetin-3-glucoside equivalent

[c]Quantified as gallic acid equivalent

Water and ethanol extracts were prepared by dissolving 20 mg of powder obtained from the extraction procedures in 1 mL of the respective solvent.

Table 6. Quantitative data for phenolic acids (hydroxycinnamic and hydroxybenzoic acids) identified in water and ethanol extracts from *Euphorbia hirta* leaves. Values are expressed as mg/g of dry extract and represent means ± standard deviation of triplicate determination (n.d. means not detected).

	Compound	Water extract (mg/g)	Ethanol extract (mg/g)
Hydroxycinnamic acids[a]			
1.1	Coumaric acid	n.d.	2.58 ± 0.07
25.1	Caffeic acid	0.26 ± 0.01	0.16 ± 0.01
34.1	Ferulic acid	1.97 ± 0.02	2.55 ± 0.06
34.2	Feruloyl-malic acid	0.52 ± 0.03	n.d.
10.1	5-*O*-coumaroyl-quinic acid *trans*	1.28 ± 0.04	1.05 ± 0.01
25.2	5-*O*-coumaroyl-quinic acid *cis*	1.25 ± 0.01	n.d.
14.1	Caffeic acid-*O*-hexoside	0.17 ± 0.01	n.d.
2.1	4-*O*-caffeoyl-quinic acid *cis*	0.15 ± 0.01	n.d.
6.5	4-*O*-caffeoyl-quinic acid *trans*	1.29 ± 0.01	n.d.
11.4	3-*O*-caffeoyl-quinic acid *cis*	0.22 ± 0.01	0.29 ± 0.01
12.1	3-*O*-caffeoyl-quinic acid *trans*	1.33 ± 0.01	n.d.
19.1	5-*O*-caffeoyl-quinic acid *trans*	7.46 ± 0.06	n.d.
23.5	5-*O*-caffeoyl-quinic acid *cis*	0.65 ± 0.05	n.d.
27.5	5-*O*-feruloyl-quinic acid	2.70 ± 0.03	n.d.
10.4	3,5-*O*-dicaffeoyl-quinic acid	0.50 ± 0.01	n.d.
5.3	Feruloyl-coniferin	3.09 ± 0.08	15.71 ± 0.24
14.4	Feruloyl-caffeoyl-quinic acid isomer	0.24 ± 0.01	n.d.
19.4	Feruloyl-caffeoyl-quinic acid isomer	0.19 ± 0.01	n.d.
	Total hydroxycinnamic acids	**23.26 ± 0.12 (14.2%)**	**22.34 ± 0.26 (45.0%)**
Hydroxybenzoic acids[b]			
13.1	Protocatechuic acid	n.d.	0.49 ± 0.01
26.1	Dihydroxy-benzoic acid	n.d.	0.21 ± 0.01
6.1	Gallic acid	1.25 ± 0.04	6.07 ± 0.28
31.3	Ethyl-gallic acid	8.96 ± 0.14	0.43 ± 0.01
10.5	Protocatechuic acid-*O*-hexoside	3.34 ± 0.02	n.d.

17.5	Digallic acid	n.d.	0.33 ± 0.01
10.2	Galloyl-shikimic acid	1.29 ± 0.02	n.d.
4.2	Galloyl-glucose isomer	0.30 ± 0.01	0.26 ± 0.01
5.2	Galloyl-glucose isomer	0.86 ± 0.02	n.d.
6.2	Galloyl-glucose isomer	0.71 ± 0.01	n.d.
8.1	Galloyl-glucose isomer	0.68 ± 0.01	n.d.
4.1	Galloyl-quinic acid isomer	1.32 ± 0.01	0.48 ± 0.01
5.5	Galloyl-quinic acid isomer	0.84 ± 0.01	n.d.
6.6	Galloyl-quinic acid isomer	0.08 ± 0.00	n.d.
25.4	Galloyl-salicin	1.04 ± 0.01	n.d.
11.2	Protocatechuic acid-*O*-pentoside-*O*-hexoside	8.03 ± 0.13	n.d.
35.2	Trigallic-acid	0.58 ± 0.01	n.d.
5.4	Galloyl-di-*O*-hexoside	0.65 ± 0.05	n.d.
15.1	Digalloyl-quinic acid	7.11 ± 0.03	n.d.
27.1	Galloyl-quinic acid-*O*-hexoside	0.49 ± 0.05	n.d.
21.3	Trigalloyl-quinic acid	5.87 ± 0.09	n.d.
	Total hydroxybenzoic acids	**43.37 ± 0.23 (26.5%)**	**8.27 ± 0.28 (16.7%)**

[a]Quantified as caffeic acid equivalent (caffeic acid derivative) or coumaric acid equivalent (coumaric acid derivative) or ferulic acid equivalent (ferulic acid derivative)

[b]Quantified as gallic acid equivalent (gallic acid derivative) or protocatechuic acid equivalent (protocatechuic acid derivative)

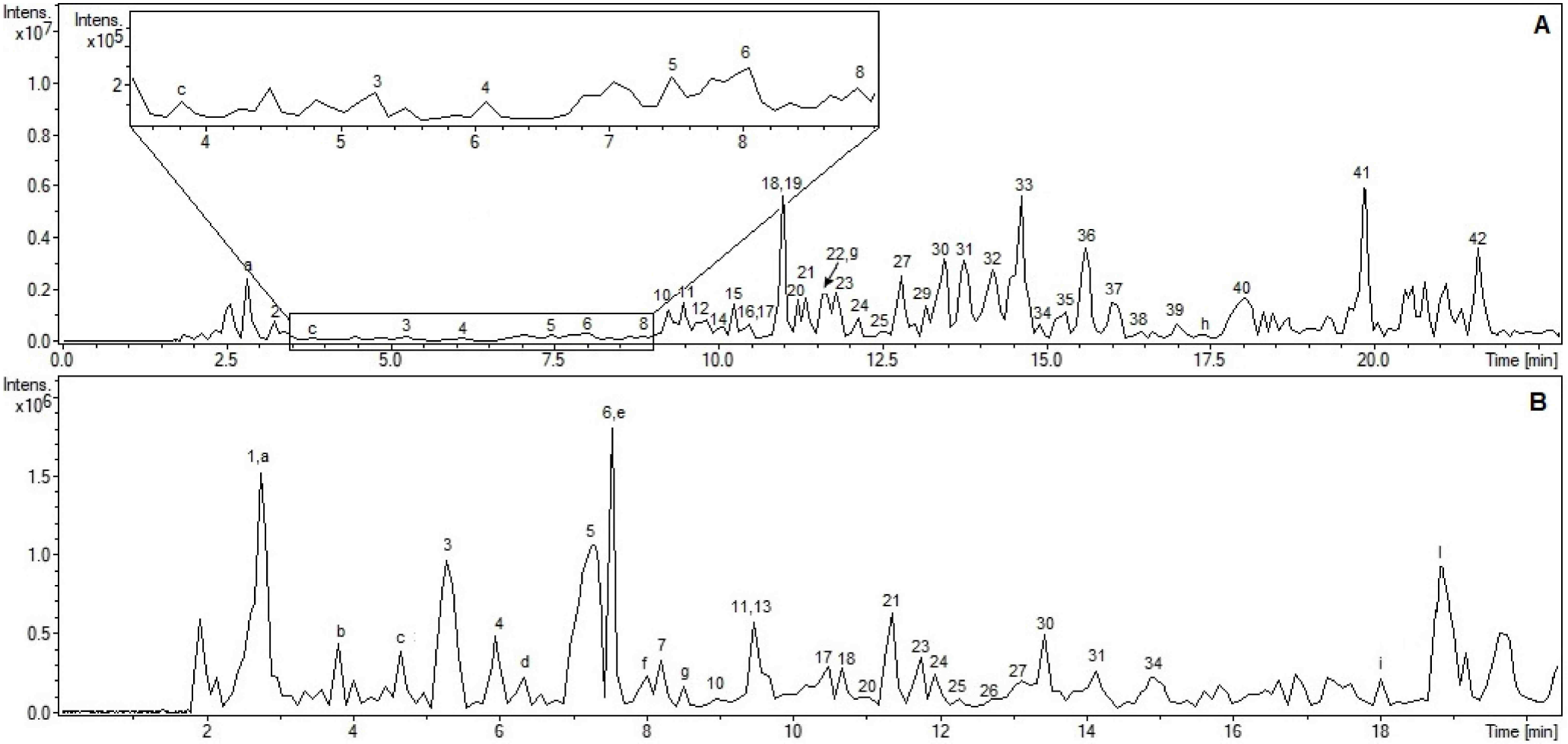
Water and ethanol extracts were prepared by dissolving 20 mg of powder obtained from the extraction procedures in 1 mL of the respective solvent.

Table 7. Mycelium growth inhibition of *Fusarium oxysporum* f. sp. *vasinfectum*, *Alternaria solani*, and *Rhizoctonia solani* as observed on potato dextrose agar medium added with the ethanol or water extracts of *Euphorbia hirta* leaves.

Extract	***Alternaria solani***	***Rhizoctonia solani***	***Fusarium oxysporum vasinfectum***
	IC_{50} (mg of dry extract/mL)		
EE	3.23 ± 0.73^{a}	3.66 ± 0.11^{a}	2.93 ± 0.14^{a}
WE	6.87 ± 0.19^{b}	32.14 ± 0.59^{b}	12.38 ± 0.21^{b}

Data are the average ± SD of five replications. Data in the same column followed by the different letters are significantly different ($p<0.05$).

WE means water extract of *Euphorbia hirta* leaves whereas EE ethanol extract of *Euphorbia hirta* leaves.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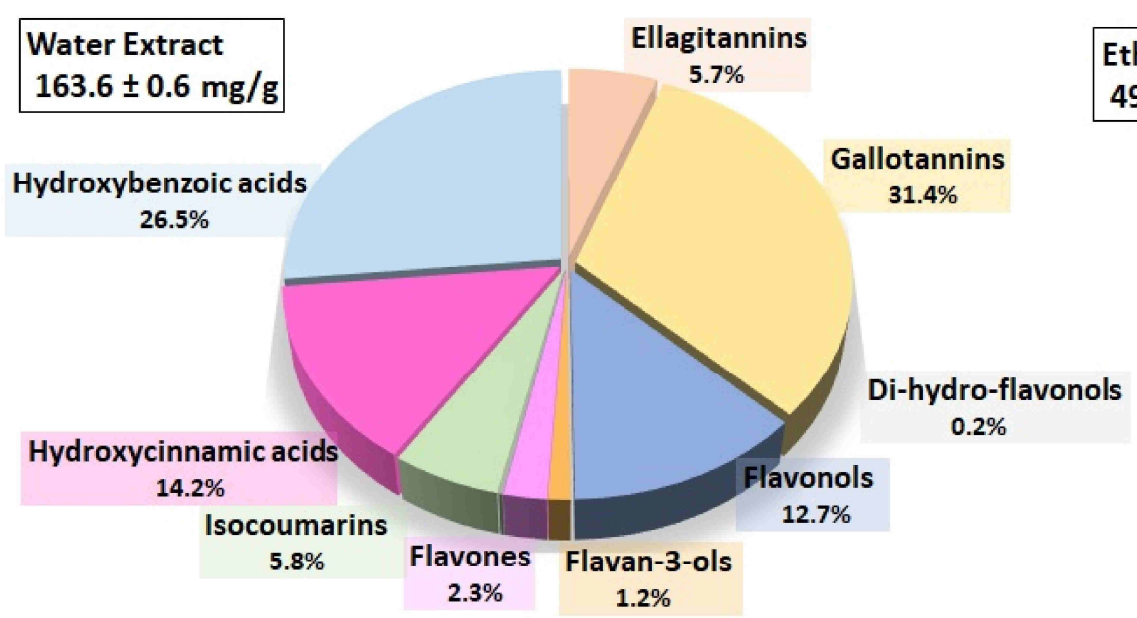
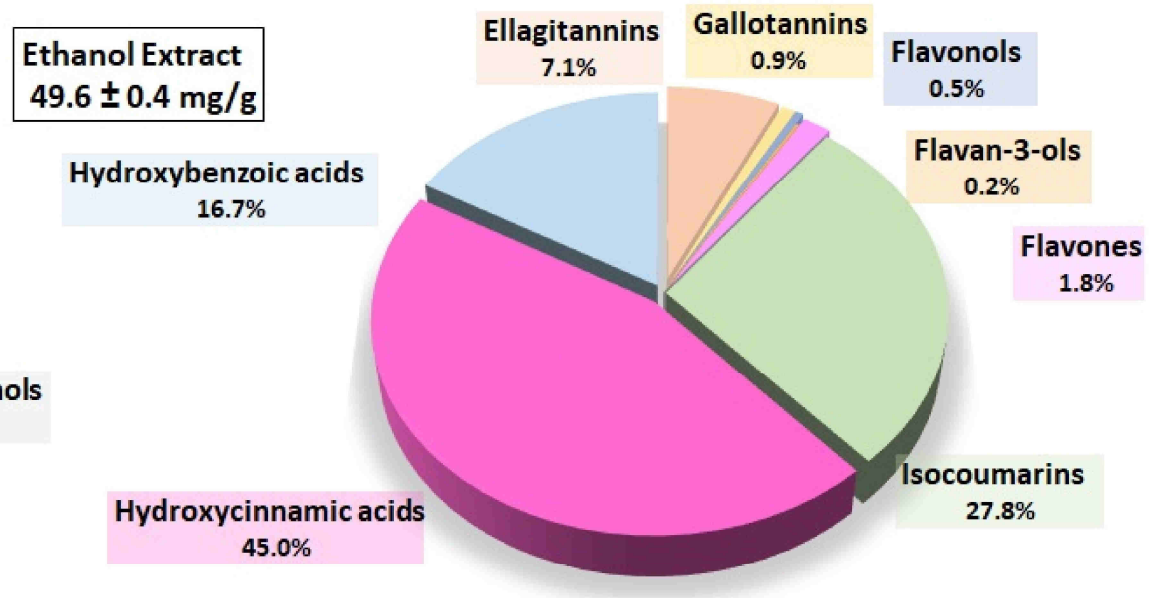
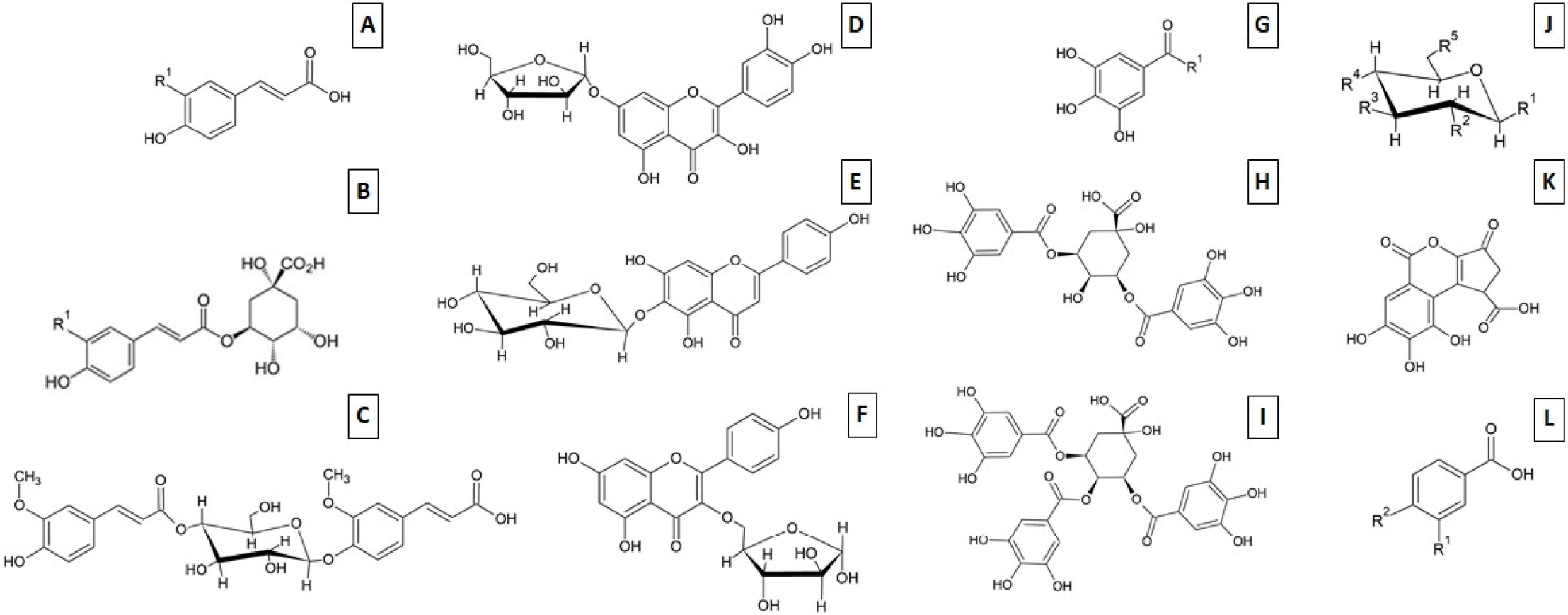
Intens.
x10^5
Time [min]
B
Intens.
x10^6
Time [min]


Water Extract
163.6 ± 0.6 mg/g
Ellagitannins
5.7%
Gallotannins
31.4%
Hydroxybenzoic acids
26.5%
Di-hydro-flavonols
0.2%
Hydroxycinnamic acids
14.2%
Flavonols
12.7%
Isocoumarins
5.8%
Flavones
2.3%
Flavan-3-ols
1.2%


Ethanol Extract
49.6 ± 0.4 mg/g
Ellagitannins
7.1%
Gallotannins
0.9%
Flavonols
0.5%
Flavan-3-ols
0.2%
Hydroxybenzoic acids
16.7%
Flavones
1.8%
Isocoumarins
27.8%
Hydroxycinnamic acids
45.0%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B
C
D
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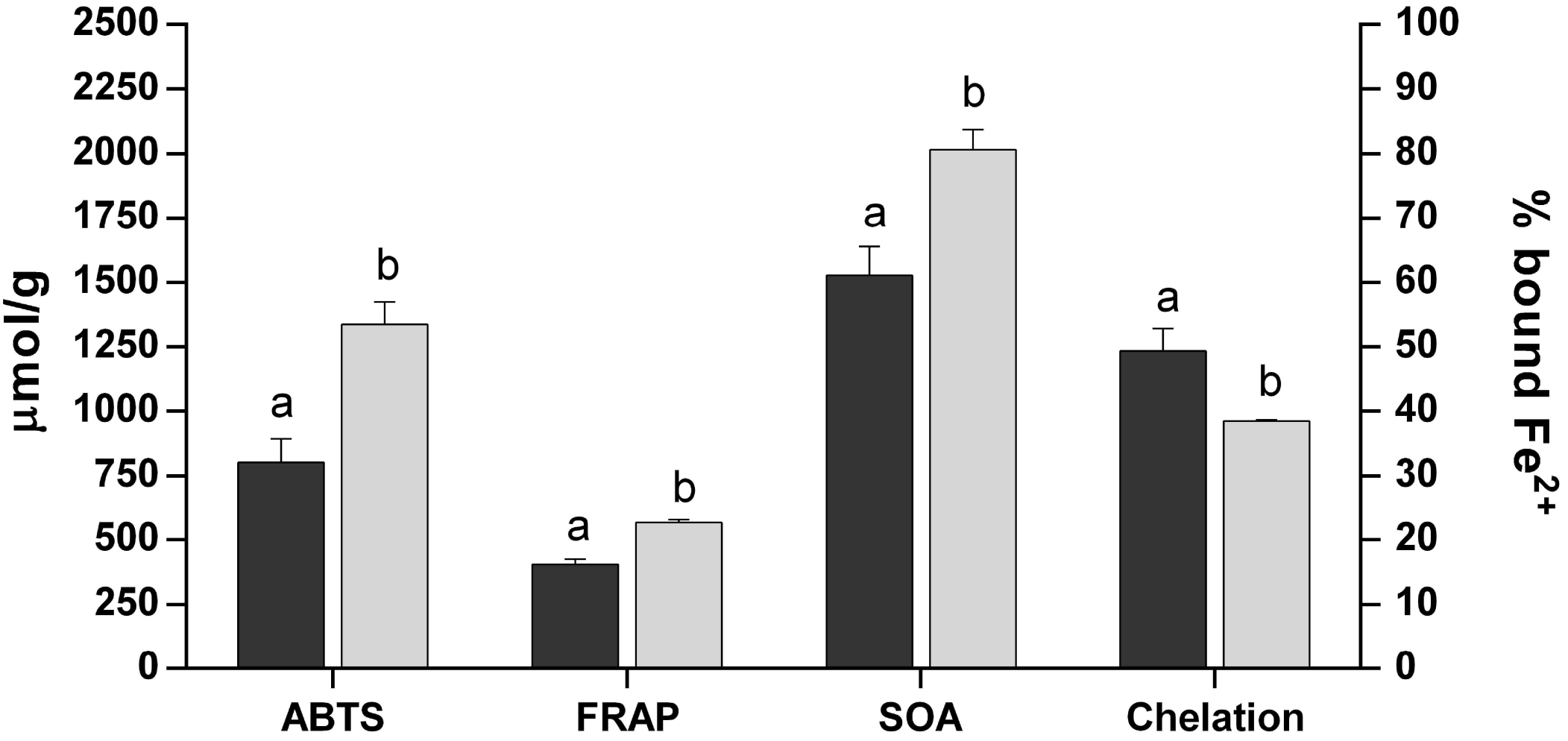
K
L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2000
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1500
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1000
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500
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50
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30
20
10
0
% bound Fe2+
a
b
a
b
a
b
a
b
ABTS
FRAP
SOA
Chelation